## REMARKS

Claims 1, 4, and 7-24 are pending in the application. Claims 2, 3, 5, 6, 25, and 26 have been canceled without prejudice. Claims 1, 4, 18, 22, and 24 have been amended. Support for these amendments can be found in the specification at, e.g., page 4, lines 21-22; page 4, lines 34-35; and page 6, line 34, to page 7, line 1. These amendments add no new matter.

35 U.S.C. §112, 1st Paragraph (Written Description)

At page 2-3 of the Office Action, the Examiner rejected claim 8 as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that that the inventors, at the time the application was filed, had possession of the claimed invention. According to the Examiner,

> Claim 8 is drawn to a polynucleotide encoding a fusion protein comprising a functionally equivalent variant of a glutathione S-transferase (GST). Therefore, these claims are drawn to a genus of polynucleotides encoding polypeptides having any structure. The specification only teaches one species, the polynucleotide encoding a fusion protein having GST. One species is not enough to describe the whole genus and there is no evidence on the record of the relationship between the structure of a polynucleotide encoding GST and the structure of a polynucleotide encoding a variant of GST. The specification also does not describe which residues of a GST are needed to impart the variant with GST activity. Therefore, the specification fails to describe a representative species of the genus of polynucleotides encoding a variant of GST having GST activity.

Applicants respectfully traverse the rejection in view of the following comments.

Claim 8 depends from claim 1 and requires that the fusion partner of the secreted fusion protein recited in claim 1 be glutathione S-transferase (GST) or a functionally equivalent variant thereof. Furthermore, the fusion partner is delimited by the functional limitation of claim 1 requiring that it enable dimerization of the soluble form of human semicarbazide-sensitive amine oxidase (SSAO).

Contrary to the Examiner's assertions in the passage above reproduced from the Office Action, claim 8 is not drawn to a genus of polynucleotides encoding polypeptides having *any* structure. Rather, claim 8 requires that the fusion partner be a GST or a functionally equivalent

variant of GST (i.e., a polypeptide that is structurally related to GST but retains the functional activity of GST). The specification details that functionally equivalent variants retain the ability to form dimers and have binding properties allowing for affinity purification (page 5, lines 24-26). Exemplary functionally equivalent variants of GST are described in the specification at, e.g., page 2, line 25 to page 3, line 3; and page 15, lines 28-29. Variants of GST can have a structure in which one or more of the cysteine residues at positions 85, 138, and 178 are replaced with other amino acid residues(s) (see, e.g., page 5, lines 24-31, and original claim 9). For example, one such functionally equivalent variant of GST (SEQ ID NO:5) can have all of the above-specified cysteine residues replaced with serine residues (see, e.g., page 5, lines 29-21; page 10, line 22 to page 11, line 21).

The written description requirement may be satisfied through disclosure of function and minimal structure when there is a well established correlation between structure and function such that one skilled in the art would be able to predict with a reasonable degree of confidence the structure of the claimed invention from a recitation of its function (MPEP §2163.II.A.3.a.i).

In addition to the above-described functionally equivalent variants of GST, applicants' disclosure as well as the publication of Tudyka and Skerra (1997) *Protein Science* 6:2180-87 ("Tudyka") (cited by the Examiner), teach that functionally equivalent variants of GST can be readily made. In addition, the publication of Lim et al. (1994) *Protein Science* 3:2233-44 (enclosed as "Exhibit A") discloses the crystal structure of GST and the amino acids which participate in the interaction of a GST dimer pair (see, e.g., pages 2237, right column, 2nd paragraph to page 2238, right column, 1st paragraph). Using the teachings of Exhibit A, which was available at the time the present application was filed, the skilled artisan would have been able to predict with a reasonable degree of confidence which structural variants of GST would retain the functional property of enabling dimerization.

In light of the above, a skilled artisan would have understood that applicants were in possession of the nucleic acid of claim 8 at the time the application was filed. In particular, the skilled artisan would have recognized that (i) there was a well established correlation between that structure of GST and the recited functional property of enabling dimerization of the soluble

form of human SSAO; and (ii) based on the above structure-function correlation, a wide spectrum of variants of GST could be readily produced, including the variants disclosed by applicants that retain the above functional property. Accordingly, applicants respectfully request that the Examiner withdraw the rejection.

35 U.S.C. § 112, 1st Paragraph (Enablement)

At pages 4-6 of the Office Action, the Examiner rejected claim 8 as allegedly not enabled. According to the Examiner,

> Claim 8 is drawn to a polynucleotide encoding a fusion protein comprising a functionally equivalent variant of a glutathione S-transferase (GST). Therefore, claim 8 encompasses polynucleotides encoding a fusion protein comprising any variants of a GST. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides encoding GST variants, broadly encompassed by the claims. Since the amino acid sequence of the encoded protein determines its structural features and properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant of modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to a polynucleotide encoding a fusion protein comprising of a GST. It would require undue experimentation of the skilled artisan to make and use the claimed variants. The specification is limited to teaching the use of a polypeptide encoding a GST but provides no guidance with regard to the making of variants and mutants or with regard to other uses. In view of the great breath of the claim, amount of experimentation required to make the claimed polynucleotides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails teach one of ordinary skill how to use the full scope of the polynucleotides encompassed by this claim.

Applicants respectfully traverse the Examiner's rejection in view of the following comments.

As detailed above in response to the written description rejection, and contrary to the Examiner's assertion, applicants provide ample guidance for making and using variants of GST that retain the functional property of enabling dimerization of a soluble form of human SSAO. Exemplary functionally equivalent variants of GST (e.g., which have cysteine residues at

positions 85, 138, and 178 substituted with other residues) are described in the specification (see, e.g., page 5, lines 24-31). In addition, the working examples describe the use of a functionally equivalent variant of GST to successfully purify a soluble form of human SSAO (see, e.g., page 10, lines 22 to page 11, lines 21; page 15, lines 20-29).

Although it is possible in certain cases to abolish the functional activity of a protein by mutating a critical amino acid residue, this does not mean that one of ordinary skill cannot nonetheless readily make functionally equivalent variants of a given protein (e.g., GST) without undue experimentation. Contrary to the Examiner statements above, the skilled artisan would have been able to readily predict the relationship between the structure of a variant of GST and the recited functional property.

As noted in Exhibit A, previous studies of GST have been "instrumental in determining the structural features of the active site essential for catalysis and substrate specificity" (see page 2234, 1st column, lines 9-12). In addition, Exhibit A teaches certain key residues that form the hydrophilic and hydrophobic interface surfaces between a GST dimer pair (see, e.g., pages 2237, right column, 2nd paragraph to page 2238, right column, 1st paragraph). Therefore, by using standard mutagenesis techniques (such as those described in the specification at page 10, lines 22 to page 11, line 18) and the teachings of Exhibit A, the skilled artisan would have been able to generate variants of GST having the requisite functional characteristic without undue experimentation and with a reasonable expectation of success.

In addition to the extensive structural information on GST that was available at the time the present application was filed, the skilled artisan would have been able to produce functionally equivalent variants of GST even by random amino acid substitutions in the GST polypeptide. As detailed in the enclosed publication of Bowie et al. (1990) *Science* 247:1306-10 (enclosed as "Exhibit B"), "proteins are surprisingly tolerant of amino acid substitutions." Exhibit B cites as evidence of this assertion a study carried out on the *lac* repressor that found that of approximately 1500 single amino acid substitutions at 142 positions in the protein, "about one-half of all substitutions were phenotypically silent." Thus, one can expect, based on Exhibit B's disclosure, that a significant percentage of random substitutions in a given protein

will result in mutated proteins with full or nearly full activity. These are far better odds than those at issue in *In re Wands,* 858 F.2d 731 (Fed. Cir. 1988), cited by the Examiner on page 4, in which the court found that screening many hybridomas to find the few that fell within the claims was not undue experimentation. The question is not whether it is possible to abolish the function of a given protein by introducing a modification, but rather whether one of ordinary skill can produce, without undue experimentation, variants in which the function is not abolished.

Based on the above comments and on the teachings of Exhibits A and B, one would predict that targeted (or even random substitutions) of amino acid residues of the GST polypeptide would result in a large pool of variants of GST that enable the dimerization of a soluble form of human SSAO. Hence, applicants submit that one of ordinary skill in the art would have been able, at the time of filing of the present application, to make and use the functionally equivalent variants of GST recited in claim 8 without undue experimentation and with a reasonable expectation of success. Accordingly, applicants request that the Examiner withdraw the rejection.

35 U.S.C. § 112, 2nd Paragraph (Indefiniteness)

At page 7 of the Office Action, the Examiner rejected claims 1 and 7-24 as allegedly indefinite. According to the Examiner, "it is not clear if the fusion protein consists of the peptides/polypeptides of (i)-(v) in the order as listed in the claim or if the fusion protein can consists of parts (i)-(v) in any order."

Claim 1 is not limited by any specific order and the use of the closed transitional phrase "consisting of" does not imply that the elements must be present in the order in which they are listed. Consistent with the foregoing, claim 1 specifies that the protease cleavage site (part (iv)) is located between the soluble form of human SSAO (part (ii)) and the fusion partner (part (iii)) and that the fusion protein may contain one or more spacer amino acid sequences (part (v)). The specification details that, for example, the "fusion partner" can be fused to the C-terminal or N-terminal portion of the soluble form of human SSAO (see, e.g., page 4, lines 34-35). Hence, the skilled artisan would understand that there are no limitations on the order of the components

of the secreted fusion protein (other than those specific limitations that are provided by the claim). The metes and bounds of the claim are not unclear merely because the components may be ordered in any of several different ways.

In view of these comments, applicants submit that claim 1 is definite and therefore respectfully request that the Examiner withdraw the rejection of claim 1.

At page 7, the Examiner rejected claim 4 as allegedly indefinite for being dependent on canceled claim 2. Claim 4 has been amended to depend from independent claim 1. In light of this amendment, applicants respectfully request that the Examiner withdraw the rejection of claim 4.

At page 5-8 of the Office Action, the Examiner rejected claim 18 as allegedly indefinite in its recitation of the phrase "derivative thereof." Claim 18 has been amended to remove this phrase, thereby obviating the rejection.

At page 8 of the Office action, the Examiner rejected claims 22-23 as allegedly indefinite. According to the Examiner,

> [i]t is not clear if the protease is fused to the fusion partner of the fusion protein recited in claim 1 or is fused to a separate fusion partner, resulting in a protease fusion independent from the fusion protein recited in claim 1 (of which claims 22-23 ultimately depend from).

Claims 1 and 22 have been amended to refer to a "first" fusion protein and a "second" fusion partner, respectively, thereby clarifying that the protease of claim 22 is fused to a separate fusion partner that is independent from the fusion protein of claim 1. Hence, amended claim 22 does not add an additional component (i.e., protease) to the fusion protein encompassed by the closed language of claim 1 (which claim 22 ultimately depends from).

In light of the claim amendments and the foregoing comments, applicants respectfully request that the Examiner withdraw the rejection of independent claim 1 and dependent claims 7-24.

35 U.S.C. §103(a) (Obviousness)

At pages 9-12 of the Office Action, the Examiner rejected claims 1, 7, 8-10, 15, 17-19, and 24 as allegedly unpatentable over Smith et al. (1998) *J. Exp. Med.* 188:17-27 ("Smith) in view of Huston et al. U.S. Patent No. 5,013,653 ("Huston") and Tudyka. According to the Examiner,

> Smith et al. (form PTO-1449 – reference AYY) teach an amino oxidase that is 100% identical to the semicarbazide-sensitive amino oxidase (SSAO) of SEQ ID NO:2 of the instant invention (Figure 1, page 20 and SwissProt sequence alignment). Smith et al. teach that the transmembrane domain is between residues 5-27 (Figure 1, page 20 and page 21). Art and the specification teach that the soluble form of SSAO lacks the membrane spanning portion of the wild-type SSAO. Even though Smith et al. teaches the transmembrane domain as including residues 5-27 of SEQ ID NO:27, one of ordinary skill in the art would have also recognized the advantage of using amino acids 29 to 763 of SEQ ID NO:2. The amino acid at position 28 is an Arg. There are numerous proteases in the cell and growth medium that cleaves at arginine residues (Huston et al. – U.S. Patent 5,013, 653, Column 10, Table 1). To ensure that the fusion partner and SSAO are not cleaved prematurely, it would have been obvious to fuse the protease cleavage site to a SSAO consisting of amino acids 29-763 of SEQ ID NO:2.

Applicants respectfully traverse the rejection in view of the following comments.

Obviousness under §103 is evaluated by considering the scope and content of the prior art, the differences between the prior art and the patent claim, the level of ordinary skill in the pertinent art, and any objective evidence of non-obviousness. Graham v. John Deere Co., 383 U.S. 1 (1966). The results of these four factual inquiries are applied to determine whether the claimed invention as a whole would have been obvious to one of ordinary skill in the appropriate art at the time the invention was made. If references must be combined or modified to realize the claimed invention, there must be some teaching or suggestion in the prior art that would have motivated one of ordinary skill to make the necessary combination or modification. In re Dillon, 919 F.2d 688, 692 (Fed. Cir. 1990) (en banc), cert. denied, Dillon v. Manbeck, 500

U.S. 904 (1991). In addition, there must be a reasonable expectation of success. The suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicants' disclosure. MPEP § 2143.

Claim 1 is directed to a nucleic acid containing a nucleic acid sequence that encodes a fusion protein consisting of: (i) a signal peptide; (ii) a soluble form of human SSAO consisting of amino acids 29 to 763 of SEQ ID NO:2 or a fragment thereof exhibiting benzylamine oxidase activity; (iii) a fusion partner that enables dimerization of the soluble SSAO; (iv) a protease cleavage site; and (v) optionally one or more spacer amino acids sequences.

Nothing in the cited references provided the skilled artisan, as of the filing of the present application, with the requisite reasonable expectation that the claimed nucleic acid could have been successfully produced. Even if a transmembrane region of a given protein has been predicted, it does not necessarily follow that a soluble form of the protein can be obtained with a reasonable expectation of success and without undue experimentation. All proteins are unique and one cannot predict whether or where a protein can be truncated to obtain an active soluble protein. Before applicants' filing of the present application, it was not known which part of SSAO, if any, and/or which expression system, if any, could be used to produce a soluble and active human SSAO. Furthermore, it was not known, which fusion partner to use, where to fuse it, which signal peptide could be used for secretion of SSAO, which linker could be used between the signal peptide and GST, or which linker could be used between GST and SSAO to be able to obtain an active protein in the growth medium.

The claimed invention permits the production, in milligram quantities, of pure, soluble and active human SSAO. Smith, Huston, and Tudyka constitute at best an invitation to vary parameters or try each of numerous possible choices until one *possibly* arrives at a successful result. However, "obvious to try" is not the standard for obviousness under §103 (see, e.g., In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed.Cir.1988); In re Geiger, 815 F.2d 686, 688, 2 USPQ2d 1276, 1278 (Fed.Cir.1987); In re Merck & Co., Inc., 800 F.2d 1091, 1097, 231 USPQ 375, 379 (Fed.Cir.1986); In re Antonie, 559 F.2d 618, 620, 195 USPQ 6, 8 (CCPA 1977).

In addition to the requirement that there must be a reasonable expectation of success, objective evidence that an invention fulfills a need that existed in the art for a long period of time without solution must also be considered under §103 (see, e.g., MPEP §716.04, I). The presently claimed invention provides a solution to the long-felt but unsatisfied need for a means of purifying to homogeneity and in high amounts a recombinant human SSAO (see, e.g., specification at page 2, lines 16-24). As evidence of this long-felt but unsatisfied need, Holt et al. (1998) *Biochemistry*, 37:4946-57 (enclosed as "Exhibit C") and Elmore et al. (2002) *J. Biol. Inorg. Chem.* 7:565-79 (enclosed as "Exhibit D") both recognized the desirability of obtaining high yields and a homogeneous supply of a mammalian copper-containing amine oxidase such as SSAO (see, e.g., Exhibit C at Abstract and Exhibit D at page 567, 1st column, lines 8-18). In addition, Exhibit D noted that the heterologous overexpression and purification of recombinant human diamine oxidase described therein was "the first successful overexpression of any mammalian copper-containing amine oxidase" (Exhibit D at Abstract and page 567, 1st column, lines 10-13) (SSAO is a mammalian copper-containing amine oxidase). Further underscoring the difficulty that persons of skill in the art had experienced in expressing and purifying copper-containing amine oxidases, Exhibit D referred to the then-recent overexpression of a copper amine oxidase from a plant species as a "notable achievement" (Exhibit D at page 574, 1st column). Accordingly, Exhibit D (which was published approximately six years after the cloning of human SSAO and approximately one year after the priority date of the present application) establishes that it had proven difficult to achieve overexpression and purification of recombinant mammalian copper-containing amine oxidases and that no such protein had been successfully expressed and purified prior to the present application. Hence, Exhibits C and D together provide objective evidence that (i) there was a long-felt need in the art for a means of producing recombinant human SSAO, and (ii) this long-felt need was not fulfilled prior to the filing of the present application. The claimed invention provides a solution to this long-felt need.

The specification describes the successful expression and purification of the secreted fusion protein encoded by the claimed nucleic acid (see, e.g., page 15, line 20, to page 16, line 18). The fusion protein was secreted from mammalian cells transfected with the claimed

nucleic acid and was purified directly from the culture medium by glutathione-affinity chromatography (see, e.g., page 16, lines 20-30). By specific proteolysis, the fusion partner (GST) and the protease were removed, providing a high yield (milligram quantities) of pure, soluble, and highly active recombinant human SSAO protein (see, e.g., page 17, line 1 to page 19, line 3), thereby satisfying the long-felt need recognized in the art.

In light of the above comments, applicants respectfully submit that the cited references do not render the claimed invention obvious and therefore request that the Examiner withdraw the rejection.

At pages 12-14 of the Office Action, the Examiner rejected dependent claim 11 as allegedly unpatentable over Smith in view of Huston, Tudyka, and Zambidis et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:5019-24 ("Zambidis"). Zambidis was cited as describing a mouse IgG1 heavy chain signal peptide.

For the reasons detailed above, Smith, Huston, and Tudyka do not provide the skilled artisan with the requisite suggestion, motivation, or reasonable expectation of success to combine the references and/or modify the references' teachings to result in the nucleic acid of claim 1. In addition, Zambidis' description of a signal peptide would not have provided a suggestion, motivation, or reasonable expectation of success to a skilled artisan to exclude the from the soluble SSAO the amino acid at position 28 and to use amino acids 29 to 763 of SEQ ID NO:2 or a fragment thereof. For at least this reason, the combination of references fails to render obvious the nucleic acid of claim 11.

At pages 14-15 of the Office Action, the Examiner rejected dependent claims 12, 13, 20, and 21 as allegedly unpatentable over Smith in view of Huston, Tudyka, and Brenda Enzyme Database, EC 3.4.22.28 ("Brenda"). Brenda was cited in the present rejection as describing 3C protease amino acid sequences.

As detailed above, Smith, Huston, and Tudyka do not provide the skilled artisan with the requisite suggestion, motivation, or reasonable expectation of success to combine and/or modify

the references' teachings to arrive at the nucleic acid of claim 1. Brenda's description of 3C protease amino acid sequences does not overcome the references' lack of suggestion (and lack of provision of a reasonable expectation of success) to construct a secreted SSAO fusion protein wherein the SSAO portion lacks amino acid position 28 and consists of amino acids 29-763 of SEQ ID NO:2 or a fragment thereof. For at least this reason, the combination of references fails to render obvious dependent claims 12, 13, 20, and 21.

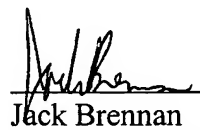
## CONCLUSIONS

Applicants ask that all claims be allowed in view of the amendments and remarks contained herein.

Enclosed is a Petition for a Two Month Extension of Time and a check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 13425-053001.

Respectfully submitted,

Date: April 18, 2005



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